Autoantibody profile and clinical patterns in 619 Italian patients with cutaneous lupus erythematosus

TITLE PAGE

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"AUTOANTIBODY PROFILE AND CLINICAL PATTERNS IN 619 ITALIAN PATIENTS WITH CUTANEOUS LUPUS ERYTHEMATOSUS"

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AUTOANTIBODY PROFILE IN CUTANEOUS LUPUS ERYTHEMATOSUS

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***Table**:3*

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ABSTRACT

Background: Anti-nuclear antibodies (ANA), anti-extractable nuclear antigens (ENA) and anti-dsDNA antibodies are often associated with cutaneous lupus erythematosus (CLE), with variable frequency depending on skin subtype. However, specific data based on large case-series on the pathogenetic, diagnostic, and prognostic meaning of such autoantibodies are still lacking.
Objective: To characterize the correlations between CLE subtypes as well as LE-non specific skin lesions and their autoantibody pattern.
Methods: Epidemiological, clinical and immunopathological data of 619 Italian patients with CLE and LE-non specific skin lesions were analyzed. Differences in age, sex, clinical features and autoantibody profile were evaluated in each LE subgroup.
Results: ANA ($p<0.0001$), anti-dsDNA ($p<0.0001$), ENA ($p=0.001$), anti-Sm ($p=0.001$), anti-RNP ($p=0.004$), anti-histone ($p=0.005$) antibodies were associated with SLE. A strong association

between ANA (p<0.0001) and anti-dsDNA (p<0.0001) and female gender was also found: positive ANA and positive anti-dsDNA had a higher prevalence among females.
Chronic CLE resulted to be negatively associated with ENA (OR=0.51, p<0.0001), anti-Ro/SSA (OR=0.49, p<0.0001) and anti-dsDNA (OR=0.37, p<0.0001). Intermittent CLE resulted to be negatively associated with ENA (OR=0.50, p=0.007) and ANA (OR=0.61, p=0.025). Subacute CLE resulted to be associated with ENA (OR=5.19, p<0.0001), anti-Ro/SSA (OR=3.83, p<0.0001), anti-Smith (OR=2.95, p=0.004) and anti-RNP (OR=3.18, p=0.007). Acute CLE resulted to be strongly associated with anti-dsDNA (OR=6.0, p<0.0001) and ANA (OR=18.1, p<0.0001).
LE-nonspecific skin lesions resulted to be significantly associated with systemic involvement. Livedo reticularis was significantly associated with ENA (p=0.007) and anti-Ro/SSA (p=0.036). Palpable purpura and periungual telangiectasia were significantly associated with ANA.
Conclusion: According to our findings, some well known associations between CLE subtypes and autoantibody profile were confirmed; moreover, specific association between autoantibodies and LE-nonspecific skin lesions were highlighted. A strict association between anti-ENA and anti-Ro/SSA antibodies and livedo reticularis, ANA and palpable purpura, and ANA and periungual teleangectasia were evidenced.

TEXT

1. INTRODUCTION

Cutaneous lupus erythematosus (CLE) is a chronic, relapsing autoimmune inflammatory disease with heterogeneous manifestations according to skin morphology, site, evolution and prognosis[1,2]. Cutaneous lesions can represent the only sign of LE and, in 23-28% of cases, can be associated with systemic involvement[3].

Based on Sontheimer and Gilliam's classification[4], cutaneous manifestations were divided into "specific and diagnostic", subclassified as chronic CLE (CCLE), subacute CLE (SCLE) and acute CLE (ACLE). Recently, the intermittent CLE (ICLE) subtype has also been introduced[5]. Among LE-nonspecific lesions of CLE, vascular lesions, diffuse non-scarring alopecia, pigmentation changes, sclerodactyly and calcinosis were included[4].

Regarding serology, anti-nuclear antibodies (ANA), anti-extractable nuclear antigens (ENA) and anti-dsDNA antibodies are often associated with several CLE subtypes[6,7].

To date, few studies have investigated the epidemiologic characteristic of CLE.

Inthe present study, we analysed the epidemiological, clinical and immunological data of LE in an Italian cross-sectional study involving patients enrolled by the Italian Group of Cutaneous Immunopathology (GIIP) during the period 2012-2015. We aimed to better characterise the specific CLE subtypes as well as LE-non specific skin lesions, evaluating the correlation between the

clinical variants of CLE and LE-non specific skin lesions with their autoantibody pattern. We also considered associated diseases.

2. MATERIALS AND METHODS

2.1 Patients

Consecutive patients with CLE were recruited from eight Lupus Clinics throughout Italy as part of a multicenter study. Demographic, clinical and laboratory data were collected at diagnosis and input into a clinical database.

The diagnosis and classification of CLE was based on clinical and histological characteristics as well as on serological parameters[9]. Four subtypes of CLE were included: CCLE [localized or generalized discoid LE (DLE), hypertrophic lupus, LE profundus/panniculitis (LEP), and chilblain LE], SCLE (papulo-squamous or annular-polycyclic variants), ACLE (localized or generalized ACLE), and ICLE. In patients with more than one CLE subtype, the form with the highest risk of developing systemic involvement was declared as the main diagnosis.

We also included SLE patients with LE specific or LE-nonspecific skin lesions, diagnosed by the presence of four or more *American College of Rheumatology* (ACR) diagnostic criteria (1982), revised in 1997[8,9].

Serological data included ANA as well as anti-dsDNA and ENA antibodies, the latter comprising anti-Ro/SSA, anti-La/SSB, anti-Sm, anti- RNP and anti-histone antibodies.

Data were compared separately between male and female patients. In the female group, a possible association between pregnancy or estrogens treatment with clinical features of CLE or LE-non specific skin lesions and autoantibody profile was also evaluated.

Finally, for each patient, comorbidities were also reported.

2.2 Statistical analysis

At baseline, differences in demographic (age and gender) clinical features (systemic/non-systemic lupus) and autoantibody profile were evaluated in each subgroup using *Fisher's exact test* for categorical variables and the *nonparametric unpaired Wilcoxon test* for continuous variables. For continuous variables, mean values with Confidence Interval 95% (CI95) were reported in the text.

A multivariate analysis by logistic regression was performed when covariates, such as age, sex or systemic/non-systemic lupus, resulted to be significantly associated with both the autoantibodies and the investigated subtypes of CLE and LE-nonspecific skinlesions.

Differences in demographic (age and gender) and clinical features (systemic/non-systemic LE, CLE subgroups) were investigatedin seven associated diseases (endocrine, respiratory, cardiovascular, gastrointestinal, oncological, rheumatic diseases, and Sjögren syndrome) using *Fisher's exact test*.

In all analyses, a 2-sided P value < 0.05 was considered statistically significant. Statistical analyses were performed using STATA software[10].

3. RESULTS

3.1 Patients

619 patients were recruited. 589 patients (95.1%) hadspecific and diagnostic manifestations of CLE at diagnosis, 30 patients (4.8%) presented with LE-nonspecific skin lesions only, and 130 patients (21.1%) featured both specific and diagnostic and LE-nonspecific lesions. A total of 160 patients (25.8%) had SLE.
The total mean age at diagnosis in all CLE patients was 45.2 ± 1.2 years. The percentage of females was 79.6% (n=493) *versus* 20.4% of males (n=126).
Results were reported in Table 1.

3.2 Autoantibodies analysis
The autoantibodies most frequently detected as positive were ANA (64.3%), followed by ENA (37.2%) and anti-dsDNA (17.9%) antibodies. Among ENA antibodies, we found the following positivity: anti-Ro/SSA (30.9%), anti-La/SSB (9.4%), anti-Sm (6.6%), anti-RNP (4.8%) and anti-histone (1%).
Concerning the associations between demographic, clinical characteristics and autoantibodies, ANA ($p<0.0001$), anti-dsDNA ($p<0.0001$), ENA ($p=0.001$), anti-Sm ($p=0.001$), anti-RNP ($p=0.004$), anti-histone ($p=0.005$) were associated with SLE. We found a strong association between ANA ($p<0.0001$) and anti-dsDNA ($p<0.0001$) and gender: positive ANA and positive anti-dsDNA had a higher prevalence among females (78.4% vs 14.4% and 37.9% vs 4.1%, respectively).

3.3 CLE subtypes analysis
CCLE was diagnosed in 48.9% (n=303) patients, divided as follows: 35.2% (n=218) localized DLE, 10.7% (n=66) generalized DLE, 2.4% (n=15) LEP, 0.6% (n=4) chilblain lupus and 0.3% (n=2) hypertrophic lupus.
SCLE was demonstrated in 18.6% (n=115) patients: 15.7% (n=97) had an annular-polycyclic SCLE and 2.9% (n=18) had a papulo-squamous SCLE.
ACLE was shown in 10.1% cases (n=63); particularly 7.9% (n=47) had a localized form of ACLE and 2.6% (n=16) had a generalized ACLE. Finally, ICLE was reported in 17.4% (n=108) patients.
Associations between autoantibodies and CLE subgroups were reported in Table2.
A systemic involvement was found in 98.4% (n=62) ACLE patients, followed by CCLE (18.5%, n=56) and SCLE (12.2%, n=14) patients. None of ICLE patients had a concomitant SLE.

3.3.1 CCLE
CCLE was diagnosed in 48.9% (n=303) patients. We found ANA positivity in 60.4% (n=183) CCLE patients; among them, 50.5% (n=110) of the patients with localized DLE and 81.8% (n=54) of the patients with generalized DLE were ANA positive. All patients with LEP (n=15) demonstrated positive ANA *versus* 63.4% of patients without LEP. All patients with chilblain LE had positive ANA as well as systemic involvement. Only two patients had hypertrophic CLE, both with positive ANA.
CCLE was negatively associated with SLE ($p<0.0001$), ENA ($p<0.0001$), anti-Ro/SSA ($p<0.0001$), anti-La/SSB ($p=0.027$), and anti-dsDNA ($p<0.0001$). Even after the multivariate logistic regression analysis, adjusting for covariates, this negative association was confirmed. CCLE resulted to be negatively associated with ENA (OR=0.51, $p<0.0001$), anti-Ro/SSA (OR=0.49, $p<0.0001$), and anti-dsDNA (OR=0.37, $p<0.0001$). Patients with CCLE had a lower prevalence of systemic

involvement (18.5 vs 32.9%), ENA (28.4 vs 45.6%), anti-Ro/SSA (22.8 vs 38.6%), anti-La/SSB (6.6 vs 12.0%), and anti-dsDNA (9.6 vs 26.0%) antibodies positivity than those without a CCLE. Analogous evidence were observed for the localized DLE while no similar associations were observed for the generalized form.

3.3.2 ICLE

ICLE was reported in 17.4% (n=108) patients. Patients with ICLE were negatively associated with ENA ($p<0.0001$) and ANA ($p<0.0001$). Even after the multivariate logistic regression analysis, adjusting for covariates significantly associated with ICLE and autoantibodies, ICLE resulted to be negatively associated with ENA (OR=0.50, p=0.007), and ANA (OR=0.61, p=0.025). Patients with *versus* those without ICLE had a lower prevalence of ENA (22.2 vs 40.3%) and ANA (43.5 vs 68.7%). None of the patients with ICLE fulfilled ACR criteria for SLE.

3.3.3 SCLE

SCLE was demonstrated in 18.6% (n=115) patients. SCLE was significantly associated with ENA ($p<0.0001$), anti-Ro/SSA ($p<0.0001$), anti-La/SSB ($p<0.0001$), anti-Sm (p=0.036), and anti-RNP (p=0.050). Even after the multivariate logistic regression analysis, adjusting for covariates significantly associated with SCLE and autoantibodies, SCLE resulted to be strongly associated with ENA (OR=5.19, $p<0.0001$), anti-Ro/SSA (OR=3.83, $p<0.0001$), anti-Sm (OR=2.95, p=0.004) and anti-RNP (OR=3.18, p=0.007).

Patients with *versus* those without SCLE had a lower prevalence of SLE (12.2 vs 29.0%), and had a higher prevalence of ENA (65.2 vs 30.8), anti-Ro/SSA (53.9 vs 25.6%), anti-La/SSB (18.3 vs 7.3%), anti-Sm (11.3 vs 5.6%), and anti-RNP (8.7 vs 4.0%). Analogous evidences emerged for the polycyclic-annular variant of SCLE. Concerning the papulo-squamous variant of SCLE, it was significantly associated with ENA (p=0.012) and anti-Ro/SSA (p=0.008). Patients with *versus* those without the papulo-squamous variant had a higher prevalence of positive ENA (66.7 vs 36.3%) and anti-Ro/SSA (61.6 vs 30.0%).

3.3.4 ACLE

ACLE was diagnosed in 10.1% of cases (n=63). ACLE was significantly associated with-SLE ($p<0.0001$), anti-dsDNA ($p<0.0001$), and ANA ($p<0.0001$). Even after the multivariate logistic regression analysis, and adjusting for sex, ACLE resulted to be strongly associated withanti-dsDNA (OR=6.0, $p<0.0001$) and ANA (OR=18.1, $p<0.0001$). ACLE had a higher prevalence of systemic involvement (98.4 vs 17.8%). All patients with ACLE but one had SLE. Analogous evidences emerged for both the localized and generalized forms of ACLE.

3.4 LE-nonspecific skin lesions analyses

The most frequently reported LE-nonspecific skin lesions were Raynaud's phenomenon (n=50, 8.1%), diffuse alopecia (n=38, 6.1%), livedo reticularis (n=23, 3.7%), urticarial vasculitis (n=19, 3.1%), palpable purpura (n=18, 2.9%), and periungual telangiectasia (n=15, 2.4%). Other LE-nonspecific skin lesions such as thrombophlebitis, anetoderma, erythema multiforme, rheumatoid nodules, sclerodactyly, calcinosis cutis and mucinosis occurred in less than 2% of the 619 patients. Associations between autoantibodies and CLE subgroups were reported in Table 3.

A systemic involvement was found in 64% of patients with LE-nonspecific skin lesions. Particularly, SLE was found in 80% (n=12) patients with periungual telangiectasia, followed by patients with urticarial vasculitis (79%, n=15), Raynaud's phenomenon (68%, n= 34), livedo reticularis (56.5%, n=13), diffuse alopecia (50%, n=19) and palpable purpura (50%, n=9).

3.4.1 Raynaud's phenomenon

Raynaud's phenomenon was found in 8.1% of patients (n=50). Raynaud's phenomenon was significantly associated with SLE (p<0.0001). Patients with *versus* those without Raynaud's phenomenon had a higher prevalence of systemic involvement (68.0 vs 22.1%).

3.4.2 Diffuse non-scarring alopecia

Diffuse non-scarring alopecia was found in 6.1% of patients (n=38). Diffuse non-scarring alopecia was significantly associatedwith SLE (p=0.001): patients with *versus* those without diffuse alopecia had a higher prevalence of systemic involvement (50.0 vs 24.3%).

3.4.3 Livedo reticularis

Livedo reticularis was found in 3.7% (n=23) of patients. Livedo reticularis was significantly associated with SLE (p=0.007), ENA (p=0.007), and anti-Ro/SSA (p=0.036). Even after the multivariate logistic regression, adjusting for systemic/non-systemic form, livedo reticularis resulted to be associated with ENA (OR=2.80, p=0.023) and anti-Ro/SSA, even if at the limit of significance (OR=2.31, p=0.053). Patients with *versus* those without livedo reticularis had a higher prevalence of systemic involvement (56.5 vs 24.7%), positive ENA (65.2 vs 36.1%) and positive anti-Ro/SSA (52.2 vs 30.0%).

3.4.4 Urticarial vasculitis

Urticarial vasculitis was found in 3.1% of patients (n=19). Urticarial vasculitis was associated with SLE (p<0.0001): patients with *versus* those without urticarial vasculitis had a higher prevalence of systemic involvement (79.0 vs 24.2%).

3.4.5 Palpable purpura

Palpable purpura was found in 2.9% of patients (n=18). Palpable purpura was significantly associated with SLE (p=0.026) and ANA (p=0.001): patients with *versus* those without palpable purpura had a higher prevalence of systemic involvement (50.0 vs 25.1%). All patients with palpable purpura had a positive ANA.

3.4.6 Periungual telangiectasia

Periungual telangiectasia was found in 2.4% of patients (n=15). Periungual telangiectasia was significantly associated with SLE (p<0.0001), and ANA (p=0.002): patients with *versus* those without periungual telangiectasia ad a higher prevalence of systemic involvement (80.0 vs 24.5%). All patients with periungual telangiectasia had a positive ANA.

3.5 drug-induced CLE

Drug-induced (DI) LE was found in 3.2% patients, ofwhom, 60% had CCLE, 30% SCLE and 10% LE-nonspecific skin lesions. DI-LE was significantly associated with anti-histone antibodies (p=0.014), while a negative association between DI-LE and anti-Ro/SSA antibodies (p=0.047) was demonstrated.

3.6 ACR criteria

Photosensitivity was found in 47.8% of patients. It was associated with ENA (p=0.027), anti-Ro/SSA (p=0.001), and anti-dsDNA (p=0.014) antibodies.

Arthritis was found in 18.9% of patients. It was associated with anti-dsDNA (OR= 4.2; p=<0.0001), ENA (p=0.003) anti-Ro/SSA (p=0.005).

Oral ulcers were present in 8.2% (n=51) of patients: 33.3% had CCLE, 22% ACLE, 15.6% SCLE and 29.1% had LE-nonspecific skin lesions);84.3% had a systemic involvement. Oral ulcers were associated with female sex (p=0.018), and anti-dsDNA (OR= 4.2; p<0.0001).

Renal disorder was found in 3.5% of patients. It was associated with SLE and anti-dsDNA (p<0.0001).

Serositis was found in 3.4% of patients. They were associated with SLE and anti-RNP (p= 0.015).

Neurologic disorder was found in 2.1% of patients. It was associated with SLE anti-dsDNA (p<0.0001).

3.7 Smoking, pregnancy and estrogens treatments

180 patients (29.6%) were smokers. A strong association between smoking and ICLE was demonstrated (p=0.002). ICLE had the highest percentage of smokers (n=46, 42.6%) in comparison with the other subtypes (CCLE 29.7%, ACLE 27.4%, SCLE 20.9%). On the contrary, a negative association between SCLE and smoking was found (p=0.024). SCLE had the lowest percentage of smokers in comparison with the other subtypes (31.6% vs 20.9%).

A strong association between smoking and CCLE patients with systemic involvement was also shown (p=0.013). Patients with CCLE and systemic involvement were smokers more often than patients with CCLE without SLE (44.6% vs 29.7%). LE-nonspecific skin lesion, followed by cutaneous small vessel leukocytoclastic vasculitis and non-scarring alopecia. All these lesions appeared in the active phases of the disease. Similar data were found in a recent study on 260 patients with SLE[32]. On thecontrary, Biazar et al. showed a higher incidence of diffuse alopecia followed by Raynaud's phenomenon. ACLE was the subtype which showed LE-nonspecific lesions more often than SCLE, but the incidence of LE-nonspecific skin lesions in ACLE was not significantly different from CCLE[11].

Smoking is considered a risk factor for CLE[33], especially for ICLE patients. In comparison with the literature data, our study showed a lower percentage of CLE smokers (29.6% vs 47.2%). We confirmed the negative influence of smoking on ICLE patients, but we added some relevant details, such as the association between smoking and CCLE patients with systemic involvement and between smoking and SLE patients with LE-nonspecific skin lesions. Thus, smoking represents a risk factor for CLE and SLE patients and smoking cessation programs should be encouraged, especially in these subgroups of patients.

Previous epidemiologic studies have shown that patients with LE have an increased risk of comorbidity[34-41]. In our study, we found an increased risk of Sjögren syndrome, as well as

endocrine and respiratory diseases in SLE patients, regardless the CLE subtypes. An association among cardiovascular and gastrointestinal diseases and age was shown; accordingly, patients with versus those without such diseases were older.

Concerning oncological diseases, it has recently been shown that patients with SCLE[42] and, in few cases, with DLE[43] have a significantly increased cancer risk, especially for oral cancer, lymphomas, respiratory cancer and non-melanoma skin cancer[44]. In our study we did not find any correlation among CLE and cancers. The only significant association was with age: elderly had a higher risk of cancers as it is shown in general population[45].

Finally, no significant associations were demonstrated among rheumatic diseases and demographic and clinical characteristics of CLE patients. However, since our study was not prospective, we collected data just at LE diagnosis and data on long-term risk about associated diseases are not available.

CONCLUSION

The present study provides important information on epidemiologic data in a cohort of 619 CLE patients. We confirmed some known associations between autoantibodies and CLE subtypes, adding some relevant details for diagnostic purposes, such as the association between anti-dsDNAantibodies and oral ulcers, regardless a systemic involvement. Since oral ulcers are often associated with SLE, we suggest to strictly monitor these patients in order to evaluate a possible systemic evolution. The same conclusions can be drawn about LE-nonspecific skin lesions, that were significantly associated with SLE.

Moreover, since smoking has a higher prevalence among CLE patients, we suggest to discuss with CLE patients its role in inducing or worsening skin and systemic lesions, in order to interrupt smoking at soon at possible.

Interestingly, our results showed that patients with SCLE had a lower prevalence of SLE compared to CCLE and ACLE subtypes. None of ICLE patients showed a systemic involvement.

Finally, patients with CLE have a higher incidence of associated diseases, such as thyroid diseases, especially in cases of systemic involvement. Thus, we suggest to evaluate all the patients with CLE to check concomitant comorbidities at the time of diagnosis.

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